CLONING OF BETA-XYLOSIDASE GENE FROM *Aspergillus niger*

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ABSTRACT

Filamentous fungi are known to be efficient producers of xylanolytic enzymes, and most commercial xylanolytic preparations are obtained from fermentations of *Aspergillus* or *Trichoderma* species. ß-xylosidase genes from *Aspergillus niger* was amplified using Polymerase Chain Reaction. ß-xylosidase gene in *Aspergillus niger* contains an open reading frame of 2,412 nucleotides which show high similarity to *Aspergillus awamori* xyl mRNA for ß-xylosidase (Accession Number: AB154359). The gene encoded 804 amino acid sequence of ß-xylosidase. Amino acid analysis shows 99% identities to ß-xylosidase from *Aspergillus awamori.*

INTRODUCTION

Plant cell walls, the major reservoir of fixed carbon in nature, contain three major polymers which are cellulose, hemicellulose and lignin. Cellulases and hemicellulases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agricultural. A great deal of interest in the enzymology of hemicellulose degradation has recently reinitiated for a number of application, most notably biofuel production and because of the biotechnological interest in the hydrolysis of hemicellulose for the pulp and paper or the feedstock industry. The major hemicellulose polymer in cereals and hardwood is xylan. Xylan consists of a ß-1,4-linked D-xylose backbone and can be substituted by different side groups such as L-arabinose, D-galactose, acetyl, feruloyl, *p*-coumaroyl, and glucuronic acid residues. The biodegradation of the xylan backbone depends on two classes of enzymes. Endoxylanases (EC 3.2.1.8) are able to cleave the xylan backbone into smaller oligosaccharides, which can then be degraded further to xylose by ß-xylosidases (EC 3.2.1.37). β-xylosidase which has been reported to be rate limiting in arabinoxylan hydrolysis is therefore important for complete hydrolysis of xylan, the most abundant hemicellulose. ß-Xylosidases are highly specific for small unsubstituted xylose oligosaccharides and their action results in the production of xylose.

MATERIALS AND METHODS

Aspergillus niger; (Bead stock, -80°C) was grown on PDA plate and harvested on the 9th using 4 ml of 1% Tween 80.

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Spore was grown in Potato Dextrose Broth for 7 days. Mycelium was filtered for the genomic DNA isolation.

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Isolation of genomic DNA was carried out using the CTAB methods by Murray and Thompson (1980).

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Isolation of Beta-xylosidase gene was carried out by PCR. Primer was designed based on the sequence in NCBI database

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The Beta-xylosidase gene was clone in puc19 vector and transformed in *E. coli* JM109.

RESULTS AND DISCUSSION

Figure1: Genomic DNA
Lane 1: lambda *Hind*III digested DNA marker
Lane 2: *Aspergillus niger* genomic DNA

The genomic DNA was extracted using CTAB methods by Murray and Thompson (1980). The DNA obtained was used as a template for amplification of beta-xylosidase gene via PCR.

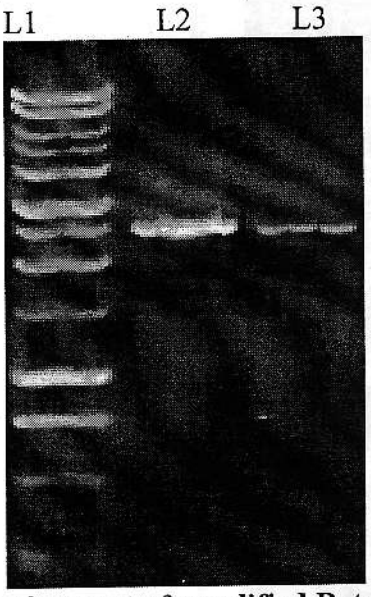

Figure 2: PCR fagment of amplified Beta-xylosidase gene
Lane 1: 1 kb DNA marker
Lane 2: Amplified beta-xylosidase gene (with signal peptide) from *Aspergillus niger*
Lane 3: Amplified beta-xylosidase gene (without signal peptide) from *Aspergillus niger*

The PCR fragment was cloned in pUC19 vector. Figure 3 shows two bands of the digested plasmid which is the puc19 vector (~2686bp) and the beta xylosidase gene (~2.5 kb) with signal peptide in Lane 2 and without signal peptide in Lane 3

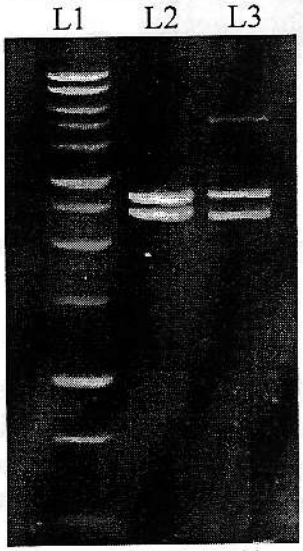

Figure 3: Recombinant plasmid after digestion on agarose gel
Lane 1: 1 kb DNA marker
Lane 2: Digested beta-xylosidase gene (with signal peptide)in puc19
Lane 3: Digested beta-xylosidase gene (without signal peptide) in puc19

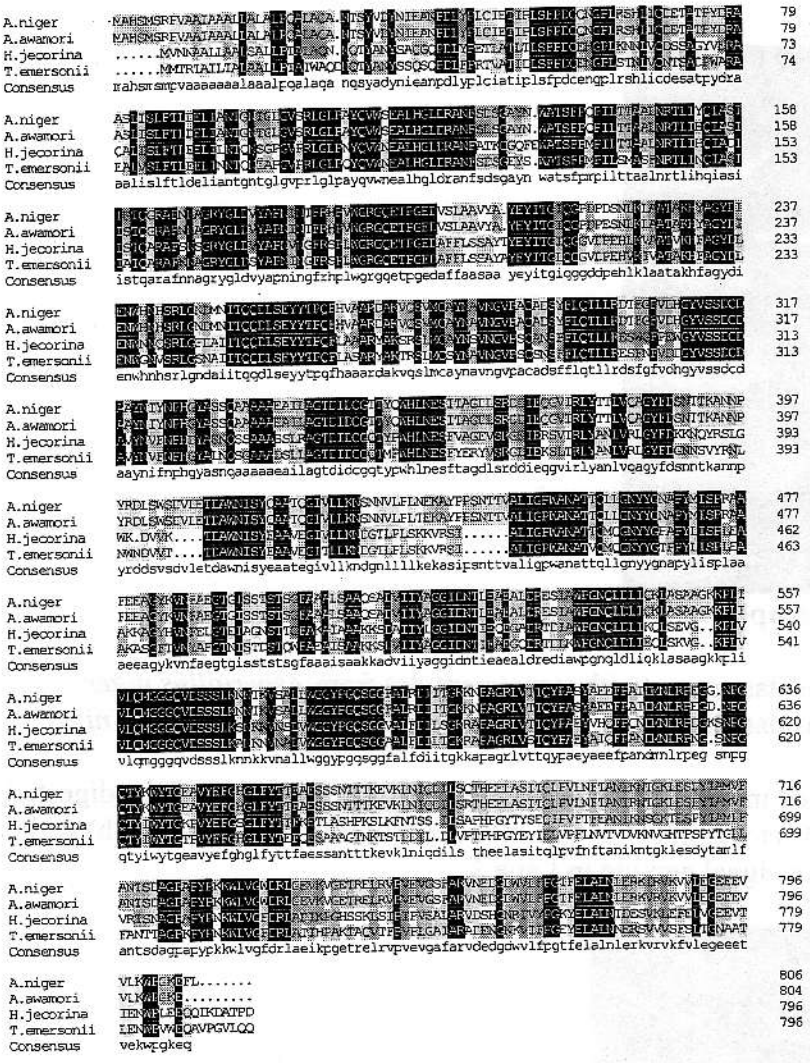

Figure 4: Amino acids sequence comparison of ß-xylosidase from various fungi.

CONCLUSION

ß-xylosidase genes from *Aspergillus niger* was amplified using Polymerase Chain Reaction. It contains an open reading frame of 2,412 nucleotides which show high similarity to *Aspergillus awamori* xyl mRNA for ß-xylosidase (Accession Number: AB154359). The gene encoded 804 amino acid sequence of ß-xylosidase. Amino acid analysis shows 99% identities to ß-xylosidase from *Aspergillus awamori.*